POTASSIUM CHANNEL OPENERS PREVENT POTASSIUM-INDUCED CALCIUM LOADING OF CARDIAC CELLS: POSSIBLE IMPLICATIONS IN CARDIOPLEGIA

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Hyperkalemic solutions that are used as cardioplegic agents, while effective in inducing electromechanical arrest, are only partially cardioprotective, and ventricular dysfunction has been observed. The underlying pathophysiology of cardioplegia-associated ventricular dysfunction is complex and not fully understood, but it could be related, in part, to intracellular Ca^{2+} loading induced by high K^+ concentrations present in cardioplegic solutions. Yet no effective cytoprotective means against possible intracellular Ca^{2+} loading, under these conditions, has been described. Recently, potassium channel openers, which open adenosine triphosphate–sensitive K^+ channels, have been reported to possess cardioprotective properties under global ischemic conditions. However, it is not known whether these novel agents could prevent intracellular Ca^{2+} loading that could occur during cardioplegia. Intracellular Ca^{2+} was monitored in ventricular myocytes, loaded with the Ca^{2+}-sensitive fluorescent probe Fluo-3AM, using epifluorescent digital imaging and laser confocal microscopy. Exposure of a myocyte to a 16 mmol/L concentration of K^+, a concentration of K^+ commonly used in cardioplegic solutions, induced a nonhomogeneous increase in intracellular Ca^{2+}. Potassium channel opening drugs, such as aprikalim or nicorandil, effectively prevented these solutions from increasing intracellular Ca^{2+}. The preventive effect of potassium channel opening drugs was antagonized by glyburide, a selective blocker of adenosine triphosphate–sensitive K^+ channels. This study demonstrates, at the single cardiac cell level, that solutions containing a 16 mmol/L concentration of K^+ promote intracellular Ca^{2+} loading, which can be prevented by potassium channel opening drugs. Therefore, potassium channel opening drugs should be considered to prevent intracellular Ca^{2+} loading associated with the use of cardioplegic solutions. (J Thorac Cardiovasc Surg 1996;112:820-31)

Cardioplegic solutions that contain high concentrations of K^+ have been used to achieve cardiac arrest and to protect the myocardium during cardiopulmonary bypass operations.[1, 2] Although effective in inducing electromechanical arrest, cardioplegic solutions are only partially cardioprotective against global surgical ischemia.[3-5] Despite various modifications implemented in hyperkalemic cardioplegic solutions, ventricular dysfunction is common and still contributes to the morbidity and mortality associated with cardiac surgery.[6-8]

The pathophysiology underlying cardioplegia-related ventricular dysfunction is complex and, at present, not fully understood.[6-8] However, myocardial cytosolic Ca^{2+} accumulation has been observed after a cardioplegic challenge and could play a role in the development of ventricular dysfunction.[9, 10] The cellular mechanism of Ca^{2+} accumulation has been related, in part, to the high K^+ concentration present in conventional cardioplegic solutions.[9]

Neither the spatial distribution of intracellular Ca^{2+} changes induced by high K^+–containing solutions nor effective cytoprotection against intracellu-

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lar Ca^{2+} loading has been defined in cardiac cells. Recently, potassium channel openers, which activate K^+ channels sensitive to adenosine triphosphate (ATP), have been shown to be cardioprotective during ischemic insults and may improve myocardial protection during global surgical ischemia.[11] However, it is not known whether potassium channel openers are capable of protecting cardiac cells from Ca^{2+} loading during a high K^+ cardioplegic challenge.

The aims of the present study were as follows: (1) to determine intracellular Ca^{2+} changes in single cardiac cells exposed to high K^+ solutions; (2) to examine whether two currently used strategies to minimize myocardial injury, namely removing extracellular Ca^{2+} or adding extracellular Mg^{2+},[12-14] affect intracellular Ca^{2+} level in cardiac cells exposed to high K^+ solutions; and (3) to evaluate whether drugs that open potassium channels protect ventricular myocytes from intracellular Ca^{2+} changes induced by high K^+ solutions.

Methods

Cell isolation. Ventricular myocytes were isolated from guinea pig hearts by enzymatic dissociation.[15] In the pentobarbital anesthetized and artificially ventilated animal, the aorta was rapidly cannulated and the heart retrogradely perfused through the coronary arteries at 37° C with the following solution: (1) normal Tyrode solution for 5 minutes to remove the blood, (2) nominally Ca^{2+}-free solution for 5 minutes to cleave desmosomal and intermediate junctions, (3) nominally Ca^{2+}-free solution containing collagenase (0.04 gm/100 ml Sigma type I, Sigma Chemical Company, St. Louis, Mo.) for 45 minutes to disrupt extracellular matrix, and (4) high K^+–low Cl^- solution for 5 minutes to remove the collagenase and provide high-energy substrate to the partially digested myocardium. So that loosened myocytes could be released, a small piece of the collagenase-treated ventricle was dissected and agitated in dishes filled with Tyrode solution in which cells then rested. Isolated cardiac myocytes were used because they are a pure myocardial preparation with no neuronal or vascular elements, and the response of isolated cells is not affected by diffusion barriers between the surface and the core of the muscle or by changes in preload, afterload, or coronary flow. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Mayo Clinic (No A33-94).

Fluorescent probes. The Ca^{2+}-selective fluorescent probe Fluo-3 acetoxymethylester (Fluo-3AM) was used to monitor relative changes in Ca^{2+} concentration. This probe exhibits lower binding capacity for Ca^{2+} and produces larger fluorescence signals after Ca^{2+} binding than conventional fluorescent probes.[16] Aliquots of 50 μg of Fluo-3AM were dissolved in 50 μl of dimethyl sulphoxide (DMSO) plus 6 μl of a 25% solution of pluronic acid. Ventricular myocytes were loaded for 45 to 60 minutes, at room temperature with Fluo-3AM (10 μmol/L). Then they were transferred to a coverlip mounted on the perforated bottom of an experimental chamber placed on the stage of an inverted epifluorescent or confocal microscope and superfused with Tyrode solution. Fluorescent measurements were carried out at room temperature (23° C). In a separate set of experiments, cardiomyocytes were loaded with the ratiometric dye Fura-2 acetoxylmethylester (Fura-2AM; 10 μmol/L) to quantify resting intracellular Ca^{2+} concentration.[17]

Epifluorescent digital microscopy. Rod-shaped ventricular myocytes with clear striation were imaged by digital epifluorescent microscopy by means of an inverted microscope (Zeiss Axiovert-135 TV, Carl Zeiss, Inc., Thornwood, N.Y.) with a 40× oil-immersion objective lens. Optimal focus was adjusted by viewing myocytes under bright field microscopy. A 100 W mercury lamp served as a source of light to excite Fluo-3AM at 488 nm (or Fura-2AM at 340 and 380 nm). Fluorescence emitted at 520 nm by the "excited" dyes was captured, after crossing a dichroic mirror, by an intensified charge-coupled device camera and digitized using the epifluorescent imaging system (Attoflor RatioVision, Atto Instruments, Inc., Rockville, Md.). Background fluorescence (Tyrode solution containing no cells) was subtracted from the fluorescence of Fluo-3AM (or Fura-2AM)–loaded myocytes.

Laser confocal microscopy. Confocal laser microscopy has made possible the precise spatial characterization of changes in intracellular Ca^{2+}. This technique permits the optical slicing of myocytes in planes as thin as 500 nm. Fluo-3AM–loaded ventricular myocytes were imaged with a Zeiss LSM-410 laser-scanning confocal microscope using the 488 nm line of an argon/krypton laser. An excitation dichroic mirror with a cutoff of 510 nm and a long-pass emission filter with a cutoff of 520 nm were used to detect Fluo-3AM fluorescence using a photomultiplier tube. Scanning optics scanned the excitation light over a sample in a raster fashion, building the image pixel by pixel. Two-dimensional confocal images were acquired by scanning an image of 100 × 100 pixels at the highest possible rate that still resulted in sufficient signal to noise. This procedure resulted in an image-acquisition rate of about 4 frames per second. Cells with detectable motion artifacts were excluded from the study. Sequences of digitized images were transferred to an SGI Indigo^2 Extreme workstation (Silicon Graphics, Mountain View, Calif.) for off-line analysis by the ANALYZE image analysis system developed at the Mayo Clinic.

Calibration of the Fura-2 and Fluo-3 signals. In cells loaded with Fura-2AM, an estimate of the Ca^{2+} concentration ($[Ca^{2+}]$) was obtained according to the equation[17, 18]:

$$[Ca^{2+}] = \frac{R - R_{min}}{R_{max} - R} K_d \beta$$

where R is the fluorescence ratio recorded from the cell and R_{min} and R_{max} represent the fluorescence ratio in the absence of Ca^{2+} (extracellular Ca^{2+} was removed and a 3 mmol/L concentration of ethylene glycol-bis(β-amino-

ethyl ether)N,N,N′,N′-tetraacetic acid [EGTA] added to the extracellular solution) and at high Ca^{2+} concentration ($CaCl_2$ 3 mmol/L), respectively. K_d is the Ca^{2+} dissociation constant of the dye (236 nmol/L), and β the ratio of minimum to maximum fluorescence at 380 nm.[17, 18] So that R_{min} and R_{max} could be obtained, Fura-2AM–loaded cardiac cells were exposed to the calcium ionophore 4-bromo A-23187. So that contraction of permealized cells exposed to high concentrations of extracellular Ca^{2+} could be prevented, myocytes were pretreated with carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (2 μmol/L) and 2,3-butaneodione monoxime (40 mmol/L). The intracellular Ca^{2+} concentration in quiescent ventricular myocytes estimated from Fura-2 AM–loaded cells was 81 ± 10 nmol/L (n = 21).

The increase in intracellular Ca^{2+} concentration as a function of Fluo-3 fluorescence was estimated by resolving the system of three equations, which include the expression for equilibrium Ca^{2+} concentration using the relative fraction of bound (f_{Ca}) and unbound (f_u) Fluo-3 to Ca^{2+}:

$$[Ca^{2+}] = K_d \frac{f_{Ca}}{f_u} \quad (1)$$

where K_d is dissociation constant of Fluo-3 (422 nmol/L) and f_{Ca} and f_u relate to the total concentration of Fluo-3 by the relation:

$$f_{Ca} + f_u = f_t \quad (2)$$

The relation between Fluo-3 intensity (F) and the above parameters is given by:

$$F = F_{max}\frac{f_{Ca}}{f_t} + F_{min}\frac{f_u}{f_t} \quad (3)$$

where F_{max} and F_{min} are the maximum and minimum Fluo-3 fluorescence intensity. Resolving equations 1, 2, and 3 relatively to Ca^{2+} concentration produces the following equation:

$$[Ca^{2+}] = K_d \frac{F - F_{min}}{F_{max} - F}$$

The estimate of cytosolic Ca^{2+} concentration is calculated taking into account the resting cytosolic Ca^{2+} concentration.[19]

Solutions and drugs. Tyrode solution had the following composition (in millimoles per liter) NaCl, 136.5; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.53; glucose, 5.5; and HEPES-NaOH, 5.5 (pH 7.4). High K^+/low Cl^- contained (in millimoles per liter) taurine, 10; oxalic acid, 10; glutamic acid, 70; KCl, 25; KH_2PO_4, 10; glucose, 11; EGTA, 0.5; and HEPES-KOH, 10 (pH 7.4). Hyperkalemic solutions were prepared by adding K^+ in a concentration of 10.6 mmol/L to the Tyrode solution (final K^+ concentration: 16 mmol/L). Hyperkalemic nominally Ca^{2+}-free solution had the same composition as the hyperkalemic Tyrode solution with the exception that Ca^{2+} was omitted. Solutions containing a 16 mmol/L concentration of $MgCl_2$ were prepared by adding $MgCl_2$ (15.5 mmol/L) to the Tyrode solution. Aprikalim, nicorandil, and glyburide were dissolved in HCl (1N), water, and DMSO, respectively, as concentrated stock solutions. All drugs were diluted to their final concentrations in the control Tyrode solution immediately before the experiment. Cardiomyocytes were incubated in Tyrode solution supplemented with aprikalim alone, nicorandil alone, or glyburide plus aprikalim for at least 10 minutes before the addition of a hyperkalemic challenge.

Statistics. Results are expressed as means ± standard error. Significance was determined by Student's t test, and $p < 0.05$ was considered significant.

Results

Hyperkalemic solutions increase intracellular Ca^{2+} concentration in cardiomyocytes. Exposure of a single ventricular myocyte to a 16 mmol/L concentration of K^+, a concentration present in commonly used cardioplegic solutions (e.g., St. Thomas' Hospital solution[1]), induced an increase in intracellular Ca^{2+} concentration (Fig. 1, *A*). Before addition of high K^+–containing solution, intracellular Ca^{2+} concentration was estimated at 133 ± 7 nmol/L (n = 74; Fig. 1, *B*), a value that is within the range for intracellular Ca^{2+} concentration measured during diastole.[20-22] On addition of a 16 mmol/L dose of K^+, intracellular Ca^{2+} concentration increased to an estimated peak value of 441 ± 32 nmol/L (n = 20; $p < 0.0001$; Fig. 1, *B*). Thus a 16 mmol/L dose of K^+ induced a 3.3-fold increase in intracellular Ca^{2+} concentration as visualized by epifluorescent microscopy in single cardiac cells.

The elevation in intracellular Ca^{2+} concentration was abrupt after the 16 mmol/L K^+ challenge, and maximal increases in intracellular Ca^{2+} concentration were reached within 15 to 20 seconds (Fig. 2, *B*). Elevation in intracellular Ca^{2+} concentration in the majority of cells was sustained at the peak level for more than 10 minutes. In some myocytes, intracellular Ca^{2+} concentration gradually declined from its peak value but remained above baseline (Fig. 2, *B*). Thus solutions containing K^+ concentrations of 16 mmol/L increase intracellular Ca^{2+} concentration above diastolic levels for a prolonged period.

Within a myocyte, high K^+–containing solutions induced an apparently nonhomogeneous spatial distribution of Ca^{2+}, with the perinuclear and central regions showing higher fluorescence than regions around the sarcolemma (Fig. 1, *A*). This may indicate a predilection for higher increases in Ca^{2+} concentration in selected subcellular anatomic regions, or it could be due to contribution of out-of-focus fluorescence coming from different optical planes.

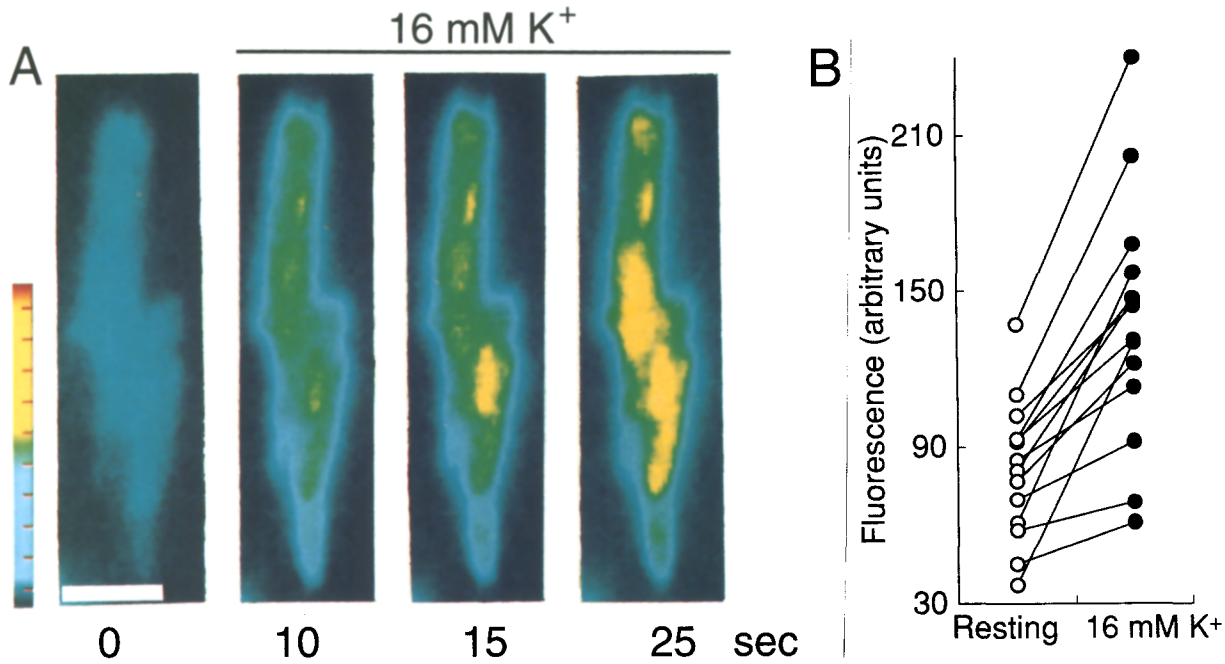

Fig. 1. Increase in intracellular Ca^{2+} induced by a hyperkalemic solution in cardiac cells. Ventricular myocytes, loaded with the Ca^{2+}-sensitive fluorescent probe, Fluo-3, were imaged by epifluorescent digital imaging. **A,** A series of four images obtained before (0 seconds) and 10, 15 and 25 seconds after raising the extracellular concentration of K^+ to 16 mmol/L. *Horizontal bar* corresponds to 20 μm. *Vertical bar* represents the Fluo-3 fluorescence displayed in pseudocolors with a continuous rainbow pattern. **B,** Fluo-3 fluorescence before (0 seconds; *open circles*) and 35 seconds after addition of K^+ *(closed circles)* recorded from ventricular myocytes obtained from 13 hearts. An estimate of the average change in intracellular Ca^{2+} concentration is given in the text.

To obtain a more precise spatial resolution of changes in intracellular Ca^{2+}, we used laser scanning confocal microscopy to record fluorescence from a single optical plane, thus effectively removing contributions of out-of-focus fluorescence. In 16 myocytes, intracellular Ca^{2+} concentration was homogeneous before exposure (estimated average concentration 121 ± 15 nmol/L) and increased by a factor of 3.5 (to an estimated concentration of 425 ± 40 nmol/L) after exposure of a cardiac cell to a 16 mmol/L concentration of K^+ (Fig. 2, *A*). The elevation in intracellular Ca^{2+} concentration was not uniform, with localized regions of higher concentration surrounded by areas of lower concentration (Fig. 2, *A*). Spatial analysis did not reveal a predilection for higher increases in fluorescence in the central region of the cell versus the perisarcolemmal area. In several myocytes, temporal analysis revealed an oscillatory pattern in the elevation of fluorescence (not illustrated). Thus scanning laser confocal microscopy confirms that changes in fluorescence induced by high K^+–containing solutions were related to nonhomogeneous elevation in intracellular Ca^{2+} concentration.

Omission of extracellular Ca^{2+} does not prevent hyperkalemic solutions from inducing intracellular Ca^{2+} loading. Extracellular Ca^{2+} has been omitted from some cardioplegic solutions (e.g., Bretschneider solution) with the expectation that this modification will prevent intracellular Ca^{2+} loading.[1] To determine whether removal of extracellular Ca^{2+} prevents changes in intracellular Ca^{2+} concentration, we exposed single myocytes to hyperkalemic, nominally Ca^{2+}-free solutions. As depicted in Fig. 3, a 16 mmol/L concentration of K^+ induced an increase in intracellular Ca^{2+} concentration, under this condition. When cardiac myocytes were exposed to hyperkalemic nominally Ca^{2+}-free solutions, intracellular Ca^{2+} concentration, was increased on average to 396 ± 50 nmol/L (n = 12), a concentration not significantly different from that obtained in solutions containing a 1.8 mmol/L concentration of extracellular Ca^{2+}. Thus omission of extracellular Ca^{2+} from hyperkalemic solutions apparently does

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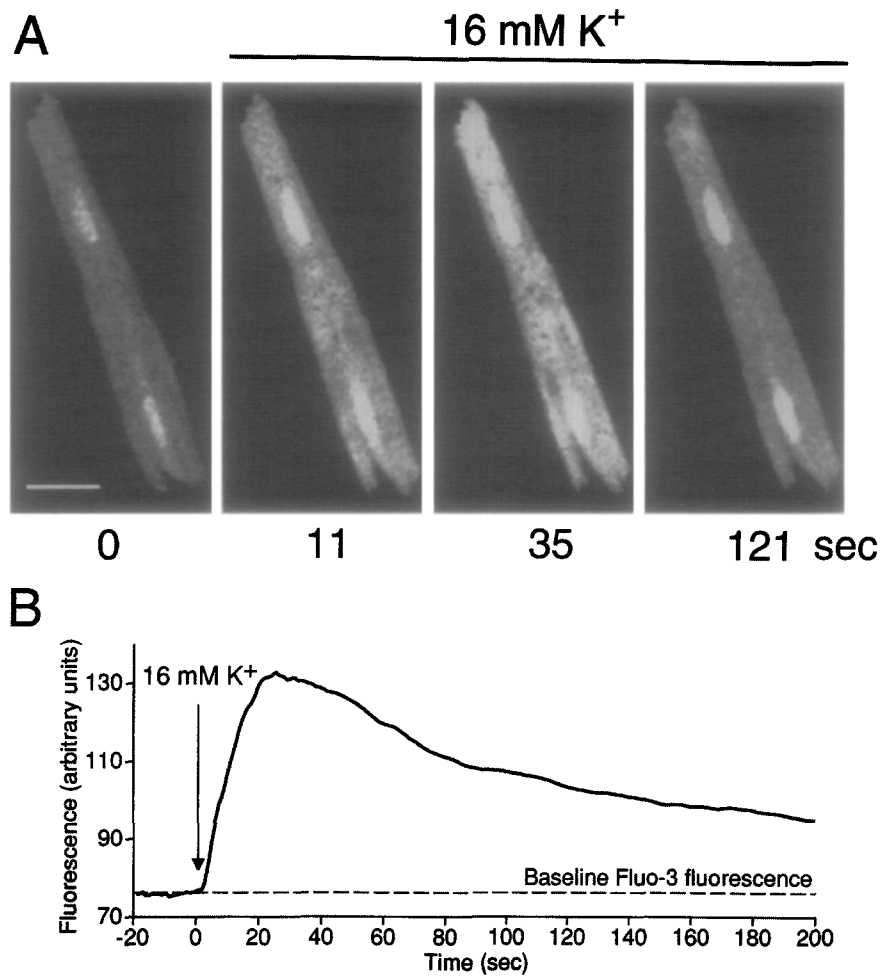

Fig. 2. Spatiotemporal distribution of intracellular Ca^{2+} revealed by laser confocal microscopy. Laser confocal microscopy was used to optically section a binucleated, Fluo-3 loaded, cardiomyocyte. **A,** Series of confocal images obtained before (0 seconds) and 11, 35, and 121 seconds after raising the extracellular concentration of K^+ to 16 mmol/L. At time 0 seconds, the baseline cytosolic fluorescence of the myocyte was low. At time 11 seconds, a nonhomogeneous increase in fluorescence occurred. At time 35 seconds, fluorescence continued to increase, making more evident the nonuniform pattern of Ca^{2+} elevation. At time 121 seconds, fluorescence decreased from its peak level but remained above baseline. Note that at all time points the fluorescence of the nuclei was higher than that of the cytosol. *Horizontal bar* represents 20 μm. **B,** Time course of changes in the average Fluo-3 fluorescence induced by raising the concentration of K^+ in the myocyte depicted in **A.** Intracellular Ca^{2+} concentration increased from an estimated 156 nmol/L (at 0 seconds) to 410 nmol/L (at 35 seconds) and 249 nmol/L (at 121 seconds).

not prevent elevation in intracellular Ca^{2+} concentration.

Extracellular Mg^{2+} does not prevent hyperkalemic solutions from increasing intracellular Ca^{2+}. Although it has been suggested that addition of a 16 mmol/L concentration of Mg^{2+} to cardioplegic solutions has a protective effect on the myocardium,[12] it is not known whether elevating extracellular Mg^{2+} prevents changes in intracellular Ca^{2+} induced by hyperkalemic solutions. Exposure of cardiac cells to solutions containing 16 mmol/L concentrations of K^+ and Mg^{2+} induced an increase in intracellular Ca^{2+} concentration (Fig. 4, *A*). Despite the presence of Mg^{2+}, 16 mmol/L, elevation in intracellular Ca^{2+} concentration was sustained (Fig. 4, *B*) and occasionally fluctuated, reflecting oscillations in intracellular Ca^{2+} concentration (Fig. 4, *B*). These oscillations may

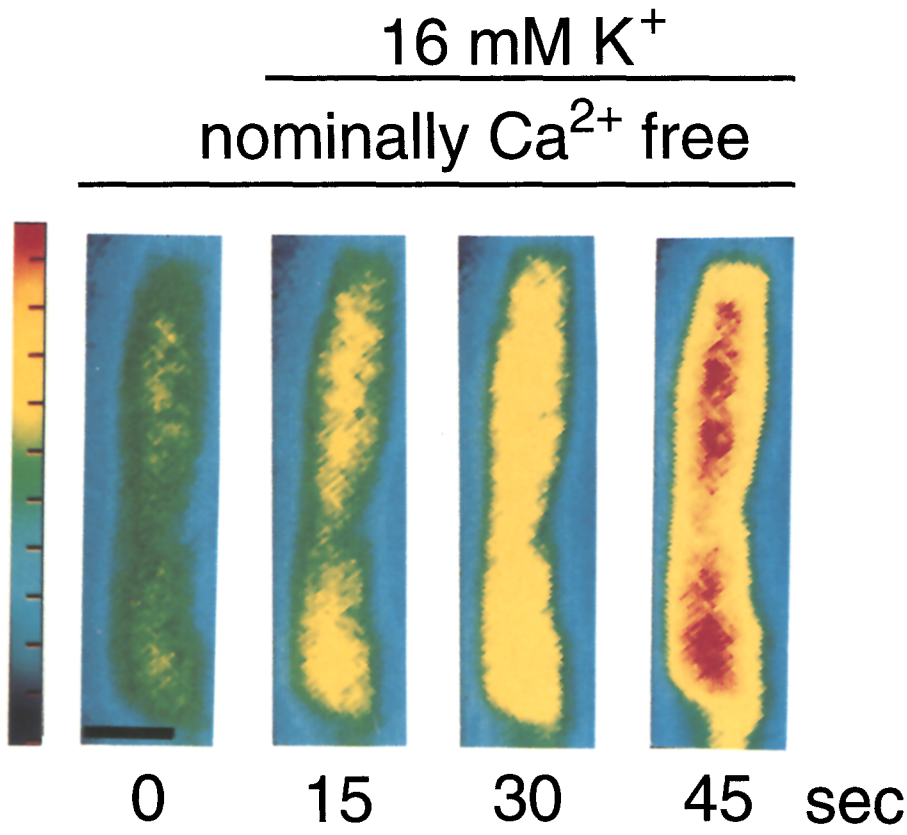

Fig. 3. Excluding extracellular Ca^{2+} does not prevent intracellular Ca^{2+} increase. A series of four epifluorescent images recorded from a ventricular myocyte incubated in nominally free Ca^{2+} solution and exposed to K^+ in a concentration of 16 mmol/L shows a time-dependent increase in Fluo-3 fluorescence from a low resting level (0 seconds) before addition of K^+ to higher levels (at 15, 30, 45 seconds) after addition of K^+. *Horizontal bar* corresponds to 25 μm. *Vertical bar* represents the Fluo-3 fluorescence displayed in pseudocolors.

reflect continuous Ca^{2+} release from and reuptake into intracellular stores. In eight myocytes (Fig. 4, *C*), the average elevation of intracellular Ca^{2+} concentration was to 463 ± 28 nmol/L, a value not significantly different from that obtained in low extracellular Mg^{2+}. Thus supplementation of hyperkalemic solutions with Mg^{2+}, 16 mmol/L, a concentration used in the St. Thomas' Hospital II cardioplegic solution, does not prevent the increase in intracellular Ca^{2+} concentration induced by a hyperkalemic challenge in ventricular myocytes.

Potassium channel opening drugs prevent hyperkalemic solutions from inducing an increase in intracellular Ca^{2+}. Potassium channel opening drugs, such as aprikalim and nicorandil, protect the myocardium from ischemic insults and modulate intracellular Ca^{2+} concentration in smooth muscle cells.[22-25] Cardiomyocytes were pretreated with aprikalim (a thioformamide) or nicorandil (a nicotinamide) before being exposed to the 16 mmol/L K^+ solution. Aprikalim (40 μmol/L) or nicorandil (300 μmol/L) prevented K^+ 16 mmol/L from inducing an increase in intracellular Ca^{2+} concentration in all 21 myocytes so tested (Fig. 5). The intracellular Ca^{2+} concentrations after addition of K^+ 16 mmol/L were 115 ± 7 nmol/L (n = 10) and 135 ± 4 nmol/L (n = 11) in aprikalim- and nicorandil-treated myocytes, respectively. Thus potassium channel opening drugs protect myocytes from hyperkalemia-induced intracellular Ca^{2+} loading.

Glyburide antagonizes the ability of a potassium channel opening drug to prevent hyperkalemia-induced elevation in intracellular Ca^{2+}. Potassium channel opening drugs activate myocardial ATP-sensitive K^+ channels.[26, 27] Cardiac cells were therefore treated with glyburide (6 μmol/L), a selective blocker of ATP-dependent K^+ chan-

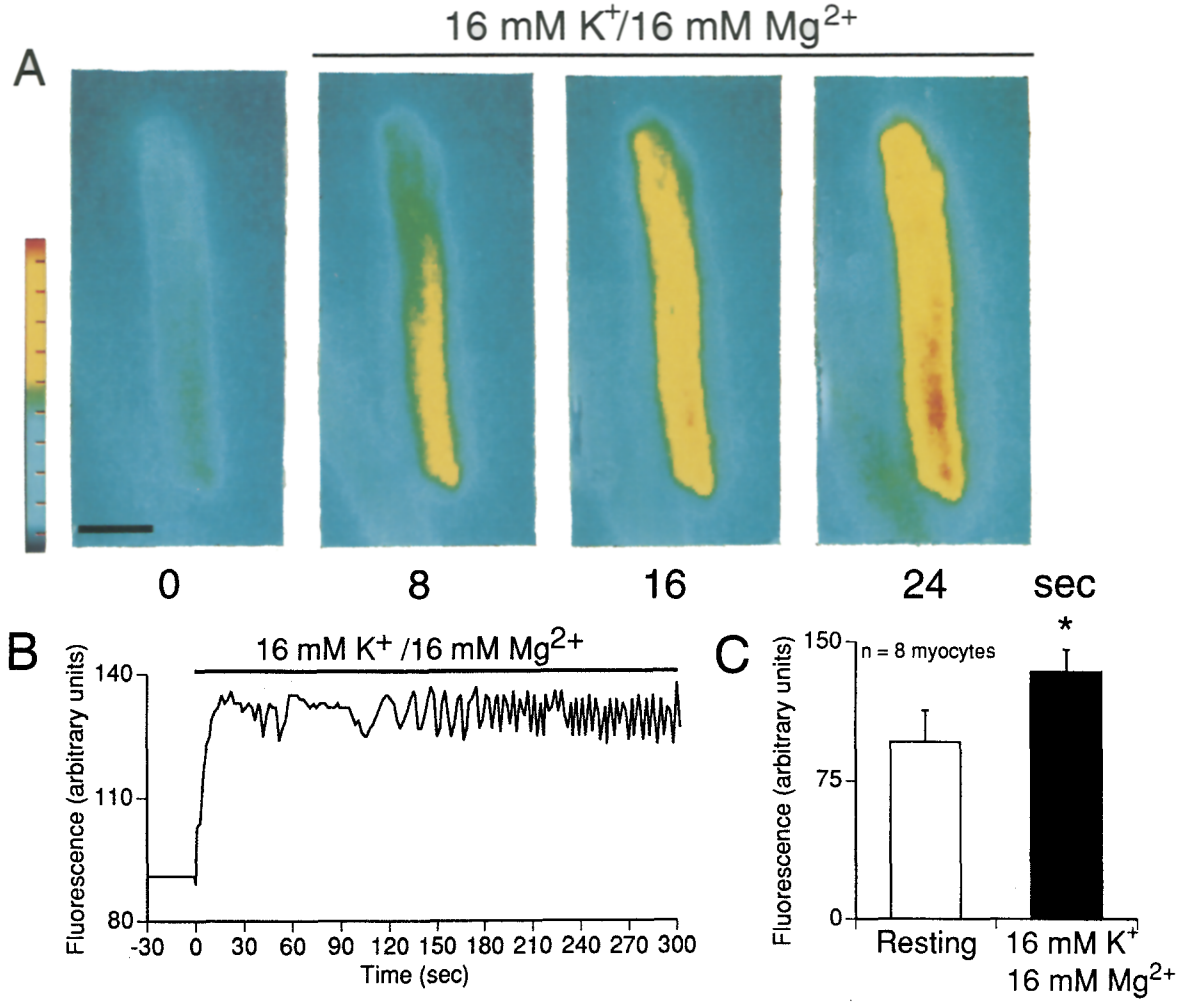

Fig. 4. Supplementing hyperkalemic solutions with $MgCl_2$, 16 mmol/L, does not prevent intracellular Ca^{2+} increase. **A,** A series of four epifluorescent images obtained from a single cardiac myocyte before (0 seconds) and 8, 16, and 24 seconds after exposure to 16 mmol/L doses of KCl and $MgCl_2$. *Horizontal bar* corresponds to 15 μm. *Vertical bar* represents the Fluo-3 fluorescence displayed in pseudocolors. **B,** Time course of relative changes in Fluo-3 fluorescence induced by 16 mmol/L doses of KCl and $MgCl_2$ in the cell depicted in **A. C,** Average fluorescence at rest and 35 seconds after exposure of eight ventricular myocytes to 16 mmol/L doses of KCl and $MgCl_2$. *Star* corresponds to $p < 0.01$. An estimate of changes in the intracellular Ca^{2+} concentration is given in the text.

nels.[28, 29] In glyburide-treated myocytes, aprikalim was unable to prevent an elevation in intracellular Ca^{2+} induced by K^+ 16 mmol/L (Fig. 6). In glyburide- and aprikalim-treated myocytes, intracellular Ca^{2+} concentration was 150 ± 6 nmol/L before and 422 ± 49 nmol/L after K^+ 16 mmol/L was added (n = 9). These results suggest that potassium channel openers prevent high K^+ from increasing intracellular Ca^{2+} through a glyburide-sensitive mechanism.

Discussion

The present study demonstrates that hyperkalemic solutions elevate intracellular Ca^{2+} concentration in isolated ventricular myocytes. These results extend previously obtained findings from multicellular heart preparations and cardiac cell suspensions[9, 10] and provide direct evidence, at the single cell level, that hyperkalemic solutions such as the one used in cardioplegia can increase intracellular Ca^{2+} concentration. An increase in intracellular Ca^{2+} concentration could

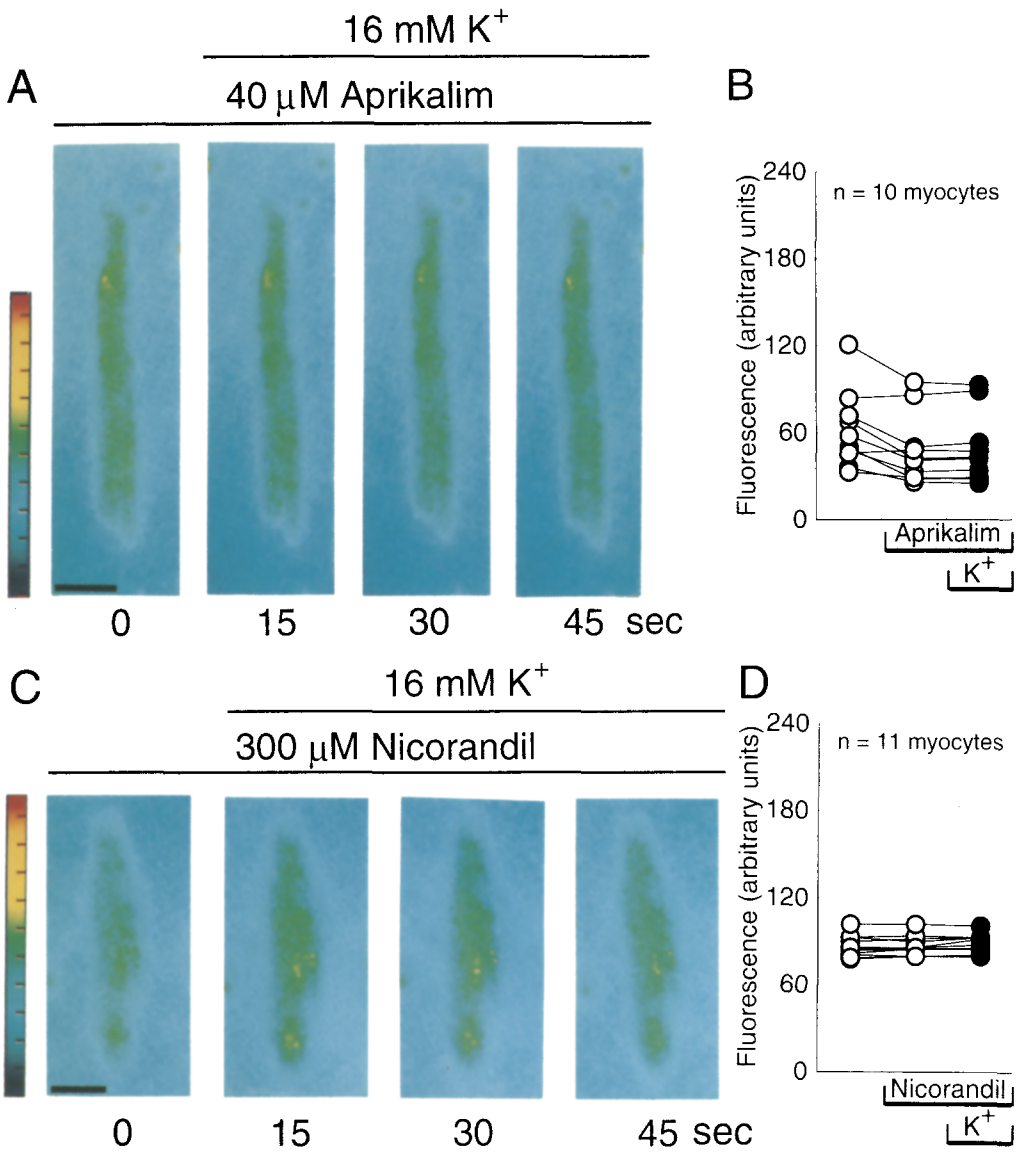

Fig. 5. Supplementing hyperkalemic solutions with potassium channel openers prevents intracellular Ca^{2+} increase. **A,** A series of epifluorescent images obtained from a single Fluo-3AM–loaded myocyte pretreated for 10 minutes with aprikalim and imaged before (0 seconds) and 15, 30, and 45 seconds after exposure to K^+, 16 mmol/L, in the continuous presence of aprikalim. **B,** Fluorescence recorded from 10 myocytes under three conditions. *Left, open circles:* baseline; *right, open circles:* in aprikalim (40 μmol/L); *closed circles:* in aprikalim and K^+, 16 mmol/L. **C,** A series of epifluorescent images of a single Fluo-3AM–loaded myocyte pretreated for 10 minutes with nicorandil and imaged before (0 seconds) and 15, 30, and 45 seconds after exposure to 16 mmol/L K^+, in the continuous presence of nicorandil. **D,** Fluorescence recorded from 11 myocytes under three conditions. *Left, open circles:* baseline; *right, open circles:* in nicorandil (300 μmol/L); *closed circles:* in nicorandil and 16 mmol/L K^+. An estimate of the changes in intracellular Ca^{2+} concentration is given in the text. *Horizontal bar* corresponds to 10 μm in **A** and **C.**

potentially lead to cellular dysfunction and contribute to myocardial damage, as previously described in various pathophysiologic conditions associated with a hyperkalemic challenge, such as cardioplegic arrest.[5]

The observed elevation in cytosolic Ca^{2+} was modest and did not lead to apparent shortening of the hyperkalemia-challenged cardiomyocyte. Yet moderate elevation of cytosolic Ca^{2+} has been related to signaling information independent of con-

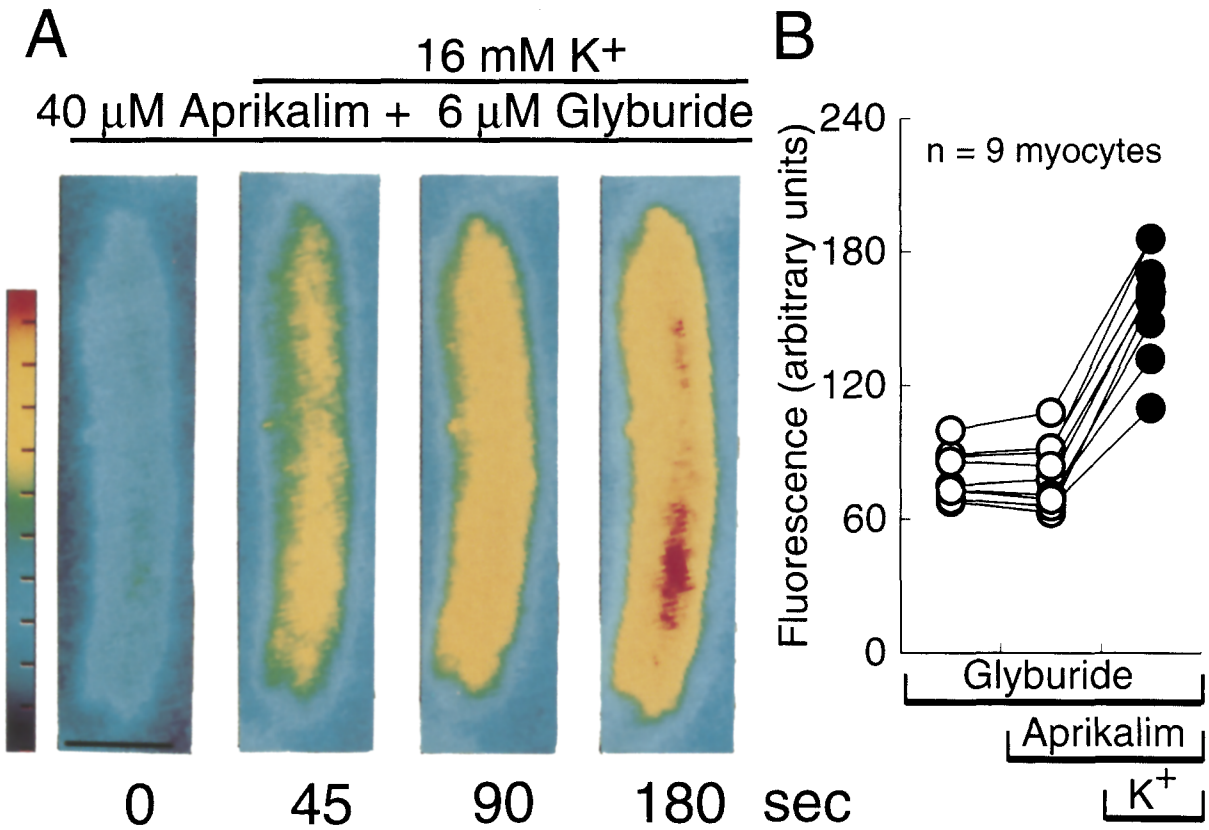

Fig. 6. A selective inhibitor of ATP-sensitive K^+ channels antagonizes the ability of a potassium channel opener to prevent hyperkalemia-induced intracellular Ca^{2+} increase. **A,** A serie of four epifluorescent images obtained from a myocyte pretreated with glyburide plus aprikalim before (0 seconds) and 45, 90, 180 seconds after addition exposure to K^+, 16 mmol/L. **B,** Summary graph depicts Fluo-3 fluorescence recorded from nine myocytes. *Circles* correspond to fluorescence recorded in individual myocytes under three different conditions. *Left, open circles:* glyburide (6 μmol/L); *right, open circles:* glyburide (6 μmol/L) plus aprikalim (40 μmol/L); *closed circles:* glyburide (6 μmol/L) plus aprikalim (40 μmol/L) and K^+ (16 mmol/L). An estimate of the changes in intracellular Ca^{2+} concentration is given in the text.

traction.[30] Because during cardioplegia the myocardium is under conditions of global ischemia with a lower production of ATP when compared with normal conditions, a modest elevation in intracellular Ca^{2+} could represent an additional load on the energy-dependent Ca^{2+}-homeostatic mechanisms and could predispose cardiac cells to reperfusion injury and diastolic dysfunction.[4, 10]

The mechanism responsible for the hyperkalemia-induced increase in intracellular Ca^{2+} concentration is most likely related to membrane depolarization,[20] because high K^+–containing solutions depolarize by at least −30 mV the cell membrane of ventricular myocytes.[31] Membrane depolarization promotes Ca^{2+} influx through voltage-dependent Ca^{2+} channels. Ca^{2+} influx, in turn, induces release of Ca^{2+} from intracellular stores.[32] Additional membrane potential–sensitive processes that regulate intracellular Ca^{2+} homeostasis may also participate in the hyperkalemia-induced increase in intracellular Ca^{2+}.[33]

Ca^{2+} has been omitted from cardioplegic solutions to limit Ca^{2+} overload during surgical ischemia.[34] However, our results with nominally Ca^{2+}-free solutions indicate that omission of Ca^{2+} from a hyperkalemic solution, as practiced with "acalcemic" cardioplegia, does not necessarily prevent intracellular increase of Ca^{2+}. This finding may suggest that in nominally Ca^{2+}-free solutions a sufficient concentration of extracellular Ca^{2+} still remains in the extracellular space and could enter during membrane depolarization to trigger release of Ca^{2+} from intracellular stores, leading to increase in intracellular Ca^{2+} concentration. Indeed, the

peak value of Ca^{2+} current at a given membrane potential is dependent on the reversal of Ca^{2+} potential which, on the basis of the Nernst equation, will not dramatically change even after reduction of extracellular Ca^{2+} to a value 10 to 20 times lower than control external Ca^{2+} concentrations. Contamination of such magnitude may be present in "acalcemic" solutions. In compromised myocytes, elevation in intracellular Ca^{2+} concentration has been ascribed to mechanisms independent of Ca^{2+} influx through Ca^{2+} channels.[35] Further studies are required to elucidate the source of Ca^{2+} leading to Ca^{2+} loading. That exposure of the heart to "acalcemic" solutions did not prevent Ca^{2+} loading is consistent with the clinical evidence showing that "acalcemic" cardioplegic solutions are not necessarily more protective than conventional cardioplegic solutions.[36]

In the myocardium, Mg^{2+} has been shown to act as a physiologic Ca^{2+} antagonist and is required as a cofactor to nucleotides for energy transfer reaction and transport processes.[37] High concentrations of Mg^{2+} in hyperkalemic cardioplegic solutions may have a beneficial effect by (1) antagonizing the unwanted effects of elevated intracellular Ca^{2+},[9, 38] (2) reducing the leakage of myocardial enzymes, and (3) preventing ATP depletion. The present study shows that adding a 16 mmol/L concentration of Mg^{2+} to high K^+ solutions did not prevent the increase in intracellular Ca^{2+}. This finding does not necessarily rule out a protective effect of Mg^{2+} on the myocardium during cardioplegic arrest independently from an increase in intracellular Ca^{2+} concentration.[13]

The major finding of the present study is that potassium channel opening drugs effectively prevent high K^+ solutions (16 mmol/L) from increasing intracellular Ca^{2+} concentration in cardiac cells. The precise mechanism of action of potassium channel openers responsible for this effect is not known. Possible mechanisms could relate to the ability of a potassium channel opener to keep the membrane potential at a more negative value when the extracellular K^+ concentration is less than 20 mmol/L.[23] It has been reported that a potassium channel opener shifts the resting membrane potential of muscle cells by approximately 15 mV to the negative direction at an extracellular concentration of K^+ close to 16 mmol/L.[23] Previously, we have demonstrated, at the whole cell and single channel level using the patch-clamp technique, that the potassium channel opening drugs used in this study, aprikalim and nicorandil, selectively activate ATP-sensitive K^+ channels in cardiac cells.[26, 27, 39] In vascular smooth muscle, K^+ channel openers appear to prevent hyperkalemic solution–induced increase in intracellular Ca^{2+} by keeping the membrane potential above the gating level of voltage-sensitive Ca^{2+} channels and preventing Ca^{2+} entry.[23] In view of the voltage-dependence of cardiac Ca^{2+} channels, a similar mechanism could be involved in underlying the reduction in Ca^{2+} influx during depolarization. At higher extracellular K^+ concentrations (above 20 mmol/L), potassium channel openers will still open K^+ channels; yet this effect will not translate into a change in the value of the resting membrane potential, since net K^+ efflux under these conditions is much less pronounced.[23] Indeed, at an extracellular K^+ concentration of 32 mmol/L, we have found no protective effect of potassium channel openers on hyperkalemia-induced Ca^{2+} loading in cardiac cells.[40] Also, potassium channel openers have been proposed to regulate intracellular Ca^{2+} handling in addition to their effect on the cellular membrane.[22, 23] Thus several mechanism(s) may underlie the effect of potassium channel openers on preventing hyperkalemia-induced Ca^{2+} loading in cardiac cells.

In various models of ischemia, opening of ATP-sensitive K^+ channels has been associated with cardioprotection.[25, 41, 42] In whole heart preparations and intact animals, aprikalim protected the myocardium from ischemic damage through a glyburide-sensitive mechanism.[25, 42, 43] The present finding, that glyburide, a selective blocker of ATP-sensitive K^+ channels,[28, 29] prevented the protective effect of aprikalim on hyperkalemia-induced intracellular Ca^{2+} increase suggests that opening of ATP-sensitive K^+ channels may play a role in protecting cardiac cells from Ca^{2+} loading.

Recently, it has been shown that potassium channel openers accelerate the recovery of myocardial function and preserve intracellular adenosine triphosphate content and mitochondrial structure after global surgical ischemia.[43-45] Under these conditions, potassium channel openers may be superior to calcium channel blockers that do not affect postischemic recovery.[46] However, the clinical use of potassium channel openers should be considered with caution because of the systemic effects, such as vasodilation, and the limited clinical experience with this novel family of therapeutics.[47] Although improved tissue selectivity of this class of compounds is an important prerequisite for the wide clinical use of

potassium channel openers, the experimental evidence that these agents could be beneficial as a supplement to hyperkalemic cardioplegic solutions during cardiopulmonary bypass are encouraging.[43-45] The findings presented herein further support such a notion because they indicate that in single cardiac cells, potassium channel openers could alleviate potentially deleterious increases in intracellular Ca^{2+} concentration associated with hyperkalemic challenges.

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